Long-term effects of nutrient and CO_2 enrichment on the temperate coral *Astrangia poculata* (Ellis and Solander, 1786)

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Abstract

Zooxanthellate colonies of the scleractinian coral *Astrangia poculata* were grown under combinations of ambient and elevated nutrients (5 µM NO_3^-, 0.3 µM PO_4^{-3}, and 2 nM Fe^{+2}) and CO_2 (~780 ppmv) treatments for a period of 6 months. Coral calcification rates, estimated from buoyant weights, were not significantly affected by moderately elevated nutrients at ambient CO_2 and were negatively affected by elevated CO_2 at ambient nutrient levels. However, calcification by corals reared under elevated nutrients combined with elevated CO_2 was not significantly different from that of corals reared under ambient conditions, suggesting that CO_2 enrichment can lead to nutrient limitation in zooxanthellate corals. A conceptual model is proposed to explain how nutrients and CO_2 interact to control zooxanthellate coral calcification. Nutrient limited corals are unable to utilize an increase in dissolved inorganic carbon (DIC) as nutrients are already limiting growth, thus the effect of elevated CO_2 on saturation state drives the calcification response. Under nutrient replete conditions, corals may have the ability to utilize more DIC, thus the calcification response to CO_2 becomes the product of a negative effect on saturation state and a positive effect on gross carbon fixation, depending upon which dominates, the calcification response can be either positive or negative. This may help explain how the range of coral responses found in different studies of ocean acidification can be obtained.

1. Introduction

As the level of CO_2 in the atmosphere and surface-ocean continues to rise, there is increasing concern about the impact of ocean acidification on corals and the diverse ecosystems they sustain. Many experimental studies have documented that scleractinian corals respond to ocean acidification and the consequent decline in aragonite saturation state (Ω) (e.g. Langdon and Atkinson, 2005), and most suggest that coral calcification decreases with decreasing saturation state. However, the saturation state of the surrounding seawater alone does not control coral calcification as corals can continue to calcify in under-saturated conditions (e.g. Marubini and Atkinson, 1999; Cohen et al., 2009) or dissolve in super-saturated conditions (e.g. Herfort et al., 2008).

In zooxanthellate corals, calcification appears tightly linked to photosynthesis by the symbiotic zooxanthellae. The median coral calcification rate in the light is three times that in the dark, and symbiotic corals calcify at higher rates than non-symbiotic corals (e.g. Gattuso et al., 1999). When corals bleach and lose their zooxanthellae, calcification rates decline (e.g. Rodrigues and Grottoli, 2006).

Photosynthesis generally responds positively to elevated CO_2 and enhanced nutrient levels. Thus, high CO_2 and high nutrient levels could enhance coral calcification. However, this is not necessarily the case. Elevated concentrations of inorganic nitrogen can increase zooxanthellae photosynthesis but cause calcification by the host coral to decline. Marubini and Davies (1996) suggested coral calcification and zooxanthellae photosynthesis compete for a limited supply of carbon. While nutrient enrichment favors growth of the zooxanthellae and increased photosynthetic activity, photosynthesis may

out-compete calcification for a limited supply of dissolved inorganic carbon (DIC), so calcification declines.

Studies of ocean acidification show that coral calcification generally declines under higher pCO_2 conditions, even in those studies where photosynthesis increases (e.g. Langdon and Atkinson, 2005). Thus, elevated CO_2 levels and elevated nutrient levels each tend to negatively affect coral calcification. However, if the CO_2 limitation model (Marubini and Davies, 1996) is correct, then the combined effects of elevated CO_2 and nutrients could be positive. Nutrient addition may enhance photosynthesis while elevated DIC could relieve the competition between photosynthesis and calcification for DIC.

To date, four studies have specifically addressed the interaction of nutrients and carbonate chemistry on calcification (Marubini and Atkinson, 1999; Marubini and Thake, 1999; Langdon and Atkinson, 2005; Renegar and Riegl, 2005). Both the work of Marubini and Thake (1999) and of Marubini and Atkinson (1999) looked only at nitrogen, yet other nutrients, such as P and Fe have been found to be limiting in many systems (e.g. Sakka et al., 1999). In three of the four studies, carbonate chemistry was manipulated by the addition of acid/base and bicarbonate. However, the effects of CO_2 may differ from those of acid addition (e.g. Jury et al., 2008). The one study to combine CO_2 and nutrients incurred high rates of coral mortality (Renegar and Riegl, 2005), which makes interpretation of calcification rates difficult.

To assess how CO_2 and nutrients (N, P, Fe) interact to affect coral calcification, this study examines a temperate coral *Astrangia poculata,* which is shown to naturally experience a wide range in aragonite saturation state. Both zooxanthellate and azooxanthellate colonies were used to examine the influence of symbiosis on the

calcification response to nutrients and CO_2. Corals were grown under long-term conditions of nutrient enrichment and reduced carbonate ion concentration, with aqueous carbonate chemistry manipulated by CO_2 enrichment.

2. Methods

2.1. Corals

Five zooxanthellate and six azooxanthellate *A. poculata* colonies were collected from the Woods Hole Oceanographic Institution (WHOI) pier near the peak summer time water temperature in early August (23.5^{o}C) at depths from 3-9 m. Specimens were returned to the lab in seawater, fragmented into four or eight pieces and trimmed of visibly bored skeleton. The fragments were attached to acrylic slides using cyanoacrylate adhesive. Once the corals were attached to the slide, the slides were suspended vertically using cable ties. Specimens were divided amongst treatments such that either one or two specimens from each parent colony were in every treatment. No mortality was observed during the course of the experiment. Corals were allowed to acclimate to aquarium conditions for 1 month, then CO_2 and nutrient levels were gradually increased over a period of 10 days to reach treatment levels.

2.2. Aquaria

Coral fragments were maintained in a flow-through aquarium system. Two 450 L reservoirs were filled daily with Vineyard Sound seawater (UV sterilized, 20 μm filtered); water from a given reservoir was continuously pumped to a header tank. From each header tank, water was fed into gas mixing chambers to equilibrate the water with a CO_2/air mixture, and from there into valve manifolds used to deliver water to individual

aquaria. Individual aquaria consisted of 1 L PET food service containers (Solo) held in 25.8±0.1^{o}C water baths (temperature controlled by an Omega CN1504 controller using aquarium heaters and thermistor temperature probes). Each container was covered with a tight fitting PET lid with 3 holes in the lid – one for water, one for air, and one for feeding. Incoming water passed through a loop of tubing inside the water bath, then dripped in from the top of the container. Outgoing water exited by a hole in the side which limited the total volume held to ~800 ml. Flow rates to each container were ~8 L/day. Each container was continuously bubbled using aquarium air-stones. Lighting was provided by three T5-HO bulbs (2 10000K bulbs, 1 actinic), on a 12 hr l/d cycle; light levels ranged from 40-115 μmol photons/m^2/s (Apogee quantum sensor), no systematic differences existed between light levels received by different treatments. Each container contained two corals, one zooxanthellate and one azooxanthellate. However, since the azooxanthellate corals acquired symbionts over the course of the experiment, no comparison of zooxanthellate and azooxanthellate colonies could be made, thus the results from the originally azooxanthellate colonies are not extensively discussed. Corals were fed daily. On alternating days, corals were fed either freshly hatched brine shrimp or frozen brine shrimp supplemented with Selcon (American Marine). Every few weeks, containers were cleaned of algal growth.

Based on previous studies in Vineyard Sound seawater (e.g. Glibert et al., 1985) dissolved inorganic nitrogen is expected to be <1 μM, and is most likely to be the limiting nutrient species. A level of 5 μM NO_3^- has been found to reduce calcification in other coral species (Marubini and Davies 1996). Thus a target enrichment of 5 μM NO_3^- was chosen for the nutrient treatment to provide a concentration which should affect

calcification and exceed the natural background level. As a precaution against inducing PO_4^{3-} or Fe limitation, both were introduced in approximate Redfield ratios based on the NO_3^- enrichment. Nutrient levels were elevated by adding $NaNO_3$, K_2HPO_4, and $FeCl_2$ stock solutions to one of the reservoirs to increase concentrations of NO_3^-, PO_4^{-3}, and Fe^{+2} by 5 μM, 0.3 μM and 2 nM respectively. Water samples were taken every few days from each reservoir. Samples were stored frozen until analyzed for ammonium, nitrate/nitrite (expressed as NO_3) and phosphate by the WHOI Nutrient Analytical Facility (using a Lachat Instruments QuickChem 8000 flow injection system). Mean levels were 3±4 μM NH_4, 6±1 μM NO_3, 0.7±0.2 μM PO_4 in the nutrient enriched treatment; ambient conditions were 3±5 μM NH_4, 0.8±0.7 μM NO_3, 0.5±0.3 μM PO_4, iron was not measured. The average difference between nutrient enriched and ambient samples collected on the same day were 0±6 μM NH_4, 5±1 μM NO_3, 0.3±0.1 μM PO_4. Average nutrient levels experienced by the corals may have been lower due to algal growth between cleanings.

CO_2 levels were increased for one set of gas mixing chambers and their corresponding individual aquaria using ambient air (provided by an air-compressor) and CO_2. Flow rates for CO_2 and air were controlled by rotameters (Alborg Instruments), to provide an air/CO_2 mixture with CO_2 levels ~400 ppm above ambient, this value was chosen to provide an enrichment greater than daily variations in aquarium pCO_2, while still being a value atmospheric pCO_2 may reach within the next 100 years (e.g. Joos et al., 1999). A Qubit s151 CO_2 analyzer and a commercially prepared CO_2 standard (Corp Brothers) were used daily to assess the stability of CO_2 levels. Average ambient CO_2 levels were 386 ±9 ppmv, CO_2 enriched levels were 780 ±40 ppmv. A dial barometer

(Fisher) was used to measure atmospheric pressure at the time of each CO_2 measurement (766±7 mmHg). Alkalinity was measured as follows: Large volume alkalinity samples were taken periodically from the WHOI pier (in Great Harbor, Woods Hole, MA) at a depth of 1.5 m starting before the start of experiments and continuing after the completion of experiments to monitor ambient conditions. A single large volume sample was taken from the water supply used to fill the reservoirs feeding the coral tanks during the course of experiments (alkalinity: 2086 µmol/kg, dissolved inorganic carbon (DIC): 1930 umol/kg, salinity: 32.02, sample taken Feb. 13, 2008). Samples were collected in 250 ml glass bottles and poisoned with 70 µl of a saturated $HgCl_2$ solution. Alkalinity and DIC were determined by closed vessel titration of ~100 ml aliquots using an automated titration system (Bradshaw et al., 1981; Brewer et al., 1986). Alkalinity and DIC concentrations were determined using a nonlinear curve fitting approach (Department of Energy, 1994), and standardized using certified reference materials obtained from Dr. A. Dickson (Scripps Institution of Oceanography).

Samples for salinity measurements were taken at the same time as large volume alkalinity samples and were analyzed using a Guildline autosal model 8400B salinometer. Small volume alkalinity samples were taken four times over the course of the experiment from the reservoirs feeding the coral tanks. Small volume samples were stored at 4^{o}C in screw cap vials (Evergreen Scientific) until analysis. Aliquots of 1 ml were used for Gran titration with 0.01 N HCl; values ranged from 2050-2150 µmol/kg, average 2110 ± 40 µmol/kg.

2.3. Buoyant weights

Buoyant weight measurements (based on Davies 1989) were made one month after the start of treatments, and again five months later. An Acculab Vicon-123 balance (accurate to 0.01 g) with weigh-below hook was used to weigh corals in ambient seawater. Glass and aluminum weights were used to estimate water density. Temperature and salinity were recorded at the same time. Corals were handled entirely underwater during buoyant weights to minimize stress or introduction of bubbles. Mass changes are assumed to reflect changes in the mass of aragonite; an aragonite density of 2.93 was used to calculate the dry weight of calcium carbonate deposited.

2.4. Alkalinity depletion

At the beginning and end of the experiment, water flow to all containers was stopped. A Ba isotope spike was added (^{135}Ba or ^{137}Ba, sufficient to double the ambient Ba concentration), and containers held without water flow for ~2 days. Small volume alkalinity samples were taken from every container at the end of the incubation to estimate calcification rates. Calcification was assumed to be the only process affecting alkalinity, with 2 moles alkalinity consumed for every mole of carbonate produced. Alkalinity samples were stored prior to measurement during which time some samples evaporated. Salinity was checked using a refractometer at the time of measurement, and samples which had evaporated during storage were excluded.

2.5. Surface area

Coral specimens were photographed using a Canon Digital Rebel XTi camera, images were saved in a raw file format. Photoshop CS3 was used to estimate the surface area of coral specimens. 2D surface areas and average diameters were determined for each specimen from images taken looking down on the coral; from side views, specimen

height was determined. The total number of polyps was also counted. In addition, average calyx diameter and depth was obtained by sectioning some of the specimens. The data presented are normalized to an estimated 3D surface area using an average radius from the 2D surface area and colony height to calculate the surface area of a cone (π x radius x height). To account for the additional area contributed from individual polyps, the polyps were assumed to add an additional conical area minus the area of the base (to avoid double counting) (π x calice radius x calice depth – π x (calice radius)^2). The average calice radius was 3.6 ± .5mm, depth 4.5±1.3mm. Individual calices are closely approximated by a cone, while coral fragments displayed considerable variability, ranging from nearly planar to roughly cylindrical, with most specimens representing a form in between planar and cylindrical; a cone was chosen as an intermediate form which could be applied to all specimens.

2.6. Statistics

A factorial ANOVA model, including the parent colony as a blocking variable, was run on rank transformed data using SAS. To make pair-wise comparisons, each treatment combination was considered a unique treatment for use in a 1-way ANOVA, Dunnett's procedure was used to compare each treatment combination to ambient; comparisons with $p<0.05$ are considered statistically significant. For each ANOVA model, residuals were plotted against predicted values, against measured values, and on normal probability plots to assess violations of ANOVA assumptions. Linear regressions were performed using Systat. All data are given as mean ± standard deviation.

3. Results

Alkalinity, DIC, salinity and temperature measurements from the *A. poculata* collection site in Woods Hole, MA are reported in Figure 1 and Supplementary Table 1. These data correspond to an annual cycle in seawater aragonite saturation state ranging from ~1.5 in the winter to ~2.5 in the summer.

Carbonate chemistry and saturation state of the seawater for each treatment, calculated from average alkalinity, salinity, phosphate and pCO_2 values are shown in Table 1. The elevated CO_2 treatments have higher bicarbonate concentrations (1840 µmol HCO_3/kg for CO_2 treatment, 1660 µmol HCO_3/kg for ambient) and lower saturation state (1.8 v 3.0) than ambient or nutrient only treatments.

Coral calcification rates determined using alkalinity depletion are compared with calcification rates determined from buoyant weight measurements in Figure 2. Data obtained by the two methods are significantly correlated ($p<0.001$), with a relationship described by the equation: buoyant weight value = 1.08* alkalinity depletion value. Alkalinity depletion measurements were made for each container, thus the values represent the combined calcification by the zooxanthellate and the originally azooxanthellate colony in each container. The alkalinity depletion value presented is the average of measurements made at the beginning and end of each treatment. To enable comparison of the alkalinity depletion and buoyant weight methods of estimating calcification, the buoyant weights of the zooxanthallate and originally azooxanthellate colonies have been combined. Since both alkalinity depletion and buoyant weight based calcification estimates show similar patterns, it is possible to compare results of different studies in which only one of the two methods has been employed.

Figure 3 shows the average calcification rates for zooxanthellate *A. poculata* colonies grown for six months under each of the nutrient and CO_2 treatments normalized to surface area. Average growth rates were 2.1±0.1, 0.7±0.1, 1.4±0.1, 1.3±0.1 g/m^2/d for ambient, CO_2 treated, nutrient treated, and CO_2 + nutrient treated corals respectively. Relative to ambient conditions, growth rates were reduced significantly by CO_2 treatment ($p<0.05$) to 34% of the ambient rate, however with the addition of nutrients, growth rates were 61%-65% of ambient regardless of CO_2 level. Table 2 shows the associated factorial ANOVA table. Treatment with CO_2 resulted in lower calcification rates ($p = 0.0212$). Nutrients alone appear to have a slightly negative effect, though this is not significant ($p = 0.7273$). However, when combined with CO_2, nutrients appear to reduce the negative effects of CO_2, suggesting a potential interaction of nutrients with CO_2, though the interaction term is not significant ($p = 0.0562$).

4. Discussion

Elevated pCO_2 levels negatively impacted calcification by *A. poculata.* However, the degree to which elevated pCO_2 impacted calcification was affected by inorganic nutrients. The decline in calcification with elevated pCO_2 at ambient nutrient levels most likely indicates a calcification response to changing carbonate chemistry (declining saturation state). Nutrient addition alone resulted in an apparent reduction in calcification of 35%, consistent with suggestions of carbon limitation under nutrient enriched conditions (e.g. Marubini and Davies, 1996). The combination of moderate nutrient addition and elevated pCO_2, however, counteracted the negative effects of elevated pCO_2 alone. This response is similar to that reported by Langdon and Atkinson (2005) for

tropical corals (Figure 4). Despite using different methodologies (HCl addition, constant DIC vs pCO_2 elevation, elevated DIC) and coral species (temperate vs tropical), both studies show that elevated nutrient conditions reduce the degree to which reduced saturation state impacts coral calcification. This suggests that coral calcification can be nutrient limited under elevated DIC conditions (or elevated $HCO_3^- + CO_2(aq)$, the two carbon species generally used for photosynthesis), while elevated nutrients can lead to DIC limitation. Thus, the response of *A. poculata* to changing pCO_2 levels depends on the nutritional status of the coral holobiont, and the response to nutrients is similarly dependent upon DIC. Conversely, Renegar and Riegl (2005) found no benefit from adding nitrate or phosphate under elevated CO_2 conditions, however, in their study, DIC was lower in the high CO_2 treatment. Similarly, Marubini and Atkinson (1999) found no benefit from adding nutrients under acidified conditions, however, their acidification treatment was also associated with lower DIC.

Based on the results reported here, and by Langdon and Atkinson (2005), it may be possible to make a general prediction as to how otherwise healthy corals could respond to elevated pCO_2 depending on the availability of nutrients or their nutritional status. Nutritionally replete corals should be able to compensate for reduced saturation state under elevated pCO_2 conditions. As pCO2 increases and seawater saturation state declines, availability of DIC to the zooxanthellae will increase, potentially allowing increased photosynthesis which provides both alkalinity and energy to help drive calcification. If the coral is experiencing carbon limitation, elevated pCO_2 could even positively impact calcification. Thus, so long as other factors such as light do not limit photosynthesis, nutritionally replete corals are expected to be relatively insensitive to

modest reductions in saturation state, and can potentially benefit from higher DIC levels. Conversely, elevation of nutrient levels may negatively impact calcification by nutritionally replete corals by increasing zooxanthellae growth which could increase photosynthesis leading to DIC limitation of calcification, or increase self shading of the zooxanthellae leading to light limitation and reduced photosynthetic efficiency (see Marubini and Davies 1996). **C**alcification by nutritionally limited corals will be strongly affected by reduced saturation state since DIC is already available at sufficient levels to support photosynthesis and calcification, thus the decline in saturation state should drive the calcification response. Nutritionally limited corals may thus respond in a manner more similar to the way inorganic aragonite precipitation responds to changing seawater saturation state (e.g., Burton and Walter, 1987). Nutritionally limited corals could benefit from modest nutrient increases so long as factors such as light availability, available space, and a lack of competition with other organisms (e.g. Lapointe, 1997) can allow an increase in nutrients to translate into increased photosynthesis and increased transfer of photosynthate to the coral host, while still having sufficient DIC to support calcification.

The interaction between nutritional status of the coral, DIC availability, and saturation state may help to explain the wide range of calcification responses seen in published acidification and nutrient enrichment studies. Figures 5 and 6 summarize data from several studies using a range of methods to manipulate carbonate chemistry and estimate calcification rates of both corals and coral communities. Only studies which provided sufficient data to calculate carbonate speciation are included. The change in coral calcification rates is presented as a function of saturation state, carbonate species, and nutrient levels. Across these studies, calcification rates were determined using one of

four methods: alkalinity depletion and buoyant weight techniques, shown in this study to be comparable (Fig. 2), and radioisotope and calcium uptake techniques, also shown to be comparable to alkalinity depletion values by Tambutte et al. (1995) and Chisholm and Gattuso (1991).

In Figure 5, all but two studies show a positive relationship between saturation state and relative calcification rate. In inorganic precipitation studies, saturation state is closely tied to precipitation rate (e.g. Burton and Walter, 1987; Holcomb et al., 2009), thus it might be expected that precipitation of aragonite by corals would show a similar dependence on saturation state, and some studies do show a similar relationship (e.g. Langdon and Atkinson, 2005; Herfort et al., 2008; the current study) (Fig. 4, 5a). However, amongst those studies showing a positive response of calcification to saturation state, the calcification response differs by orders of magnitude. Such high levels of variability suggest that saturation state alone is not an effective predicator of coral calcification. Calcification rate responses to carbonate ion follow a similar pattern to the response to saturation state (Fig. 6a). For bicarbonate (Fig. 6b), aqueous CO_2 (Fig. 6c), and DIC (Fig. 6d) some studies show positive relationships between calcification and bicarbonate, aqueous CO_2, or DIC, while others show negative relationships. Such a wide range of responses suggests that no carbonate system parameter alone can be used as an effective predictor of coral calcification.

In studies which have examined the effects of feeding, nutrients, light, and temperature without manipulating carbonate chemistry, calcification rates changed substantially in the absence of substantial changes in seawater saturation state (e.g. Marubini and Davies, 1996; Reynaud et al., 2003; Houlbreque et al., 2004; Tanaka et al.,

2007). Such results point to the importance of multiple factors in determining rates of coral calcification.

It has been suggested that bicarbonate may be the carbonate species controlling calcification rates (Herfort et al., 2008; Jury et al., 2008). However, Figure 6b, 6c, and 6d show no clear correlation between bicarbonate, aqueous CO_2, or DIC and calcification. Most of the studies showing positive calcification rate responses to elevated bicarbonate, aqueous CO_2, or DIC are studies in which saturation state is positively correlated with bicarbonate, aqueous CO_2, or DIC. Thus saturation state may be the controlling factor. The work of Reynaud et al. (2003) done at 25^{o}C and Anthony et al (2008) done at ~28.5^{o}C are exceptions, in which saturation state and calcification are not correlated. Anthony et al. (2008) used three CO_2 levels, only the highest of which was associated with a decline in calcification. Both of these studies manipulated carbonate chemistry by use of CO_2, and DIC increases as saturation state declines. These studies may represent nutrient replete conditions in which DIC is limiting calcification, thus a moderate elevation of DIC allows calcification to increase despite the decline in saturation state.

This study shows that elevated pCO_2 negatively impacts calcification, but that modest additions of nutrients (N, P, Fe) can reduce that impact. The interactions amongst 'nutrients', DIC, and saturation state yield results different from those seen when any one variable is manipulated. Aquarium-based experiments can therefore produce a wide range of coral responses to changes in seawater saturation state, all or none of which may be applicable to reef communities. In the studies discussed, only a small handful of the variables known to affect calcification and/or photosynthesis have been tested in combination with other variables, but how representative the specific values used may be

of natural reef environments is unknown. Additional complications arise in aquarium systems with fluid flow which may be much lower than encountered in a reef environment, creating diffusive boundary layers which could artificially limit transport of ions to the coral, or removal of metabolic wastes thus altering the apparent sensitivity of corals to nutrient, DIC and saturation state changes. Aquarium experiments also fail to capture how reefs, which contain countless species, will respond to changes in nutrients and CO_2 due to shifts in species abundance – macroalgae in particular are one group of organisms commonly artificially controlled in aquarium environments which may well benefit from higher nutrient levels and lead to a shift in community composition. Further laboratory-based work is needed to better understand how nutrients and/or enhanced nutritional status impact the calcification response to ocean acidification, and to identify measurable parameters, such as tissue composition, C:N:P ratios, etc. that may predict the 'nutritional' status of corals in natural settings. Ultimately, manipulation of carbonate chemistry in natural reef environments in needed to accurately quantify the coral calcification response to elevated CO_2.

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References

Anthony, K.R.N., Kline, D.I., Diaz-Pulido, G., Dove, S., Hoegh-Guldberg, O., 2008. Ocean acidification causes bleaching and productivity loss in coral reef builders. Proc Natl Acad Sci USA 105, 17442-17446.

Bradshaw, A.L., Brewer, P.G., Shafer, D.K., Williams, R.T., 1981. Measurements of total carbon dioxide and alkalinity by potentiometric titration in the GEOSECS program. Earth Planet Sci Lett 55, 99-115.

Brewer, P.G., Bradshaw, A.L. Williams, R.T. 1986. Measurements of total carbon dioxide and alkalinity in the North Atlantic Ocean in 1981. In: Reichle, D. (Ed.), The Global Carbon Cycle: Analysis of the Natural Cycle and Implications of Anthropogenic Alterations for the Next Century, Springer-Verlag, New York, pp. 358-381.

Burton, E.A., Walter, L.M., 1987. Relative precipitation rates of aragonite and Mg calcite from seawater: Temperature or carbonate ion control? Geology 15, 111-114.

Chisholm, J.R.M., Gattuso, J.-P., 1991. Validation of the alkalinity anomaly technique for investigating calcification and photosynthesis in coral reef communities. Limnol. Oceanography 36, 1232-1239.

Cohen, A.L., McConnaughey, T.A., 2003. Geochemical perspectives on coral mineralization. Reviews in Mineralogy Geochem: Biomineralization 54, 151-187.

Cohen, A. L., McCorkle, D.C., de Putron, S., Gaetani, G.A., Rose, K.A. 2009. Morphological and compositional changes in the skeletons of new coral recruits reared in acidified seawater: Insights into the biomineralization response to ocean acidification, Geochem. Geophys. Geosyst. 10, Q07005, doi:10.1029/2009GC002411.

Davies PS (1989) Short-term growth measurements of corals using an accurate buoyant weighing technique. Marine Biol. 101:389-395

Dickson, A.G. 1990. Standard potential of the reaction: AgCl(s) + 1/2 H2(g) = Ag(s) + HCl(aq), and the standard acidity constant of the ion HSO4- in synthetic seawater from 273.15 to 318.15 K. J. Chem. Thermodynamics 22:113-127.

Dickson, A. G., and F. J. Millero. 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep-Sea Research 34:1733-1743.

DOE, eds, Dickson, A.G., Goyet, C., 1994. Handbook of Methods for the Analysis of the Various Parameters of the Carbon Dioxide System in Sea Water version 2. ORNL/CDIAC-74.

Gattuso, J.P., Allemand, D., Frankignoulle, M., 1999. Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: a review of interactions and control by carbonate chemistry. Am Zool 39, 160-183.

Glibert, P.M., Dennett, M.R., Goldman, J.C. (1985) Inorganic carbon uptake by phytoplankton in vineyard sound, massachusetts. I. Measurements of the photosynthesis-irradiance response of winter and early-spring assemblages. J. Exp. Mar. Biol. Ecol. 85:21-36

Goreau, T.F., 1961. On the relation of calcification to primary productivity in reef building organisms. In: Lenhoff, H.M., Loomis, W.H. (Ed), The biology of hydra and some other coelenterates pp. 269-285.
Herfort, L., Thake, B., Taubner, I., 2008. Bicarbonate stimulation of calcification and photosynthesis in two hermatypic corals. J Phycol 44, 91-98.
Holcomb, M., Cohen, A.L., Gabitov, R.I., Hutter, J.L., 2009. Compositional and morphological features of aragonite precipitated experimentally from seawater and biogenically by corals. Geochim Cosmochim Acta 73, 4166-4179.
Houlbreque, F., Tambutte, E., Allemand, D., Ferrier-Pages, C., 2004. Interactions between zooplankton feeding, photosynthesis and skeletal growth in the scleractinian coral Stylophora pistillata. J. Exp. Biol. 207, 1461-1469.
Joos F, Plattner GK, Stocker TF, Marchal O, Schmittner A (1999) Global warming and marine carbon cycle feedbacks on future atmospheric CO2. Science 284: 464-467
Jury, C., Whitehead, R., Szmant, A., 2008. Aragonite saturation state and seawater pH do not predict calcification rates of the reef-building coral Madracis mirabilis. 11th ICRS Fort Lauderdale, FL
Langdon, C., Atkinson, M.J., 2005. Effect of elevated pCO2 on photosynthesis and calcification of corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. J. Geophys. Res. 110.
Langdon, C., Takahashi, T., Sweeney, C., Chipman, D., Goddard, J., Marubini, F., Aceves, H., Barnett, H., Atkinson, M.J., 2000. Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef. Global Biogeochem Cycles 14, 639-654.
Langdon, C., Wallace S. Broecker, Douglas E. Hammond, Edward Glenn, Kevin Fitzsimmons, Steven G. Nelson, Tsung-Hung Peng, Irka Hajdas, Bonani, G., 2003. Effect of elevated CO2 on the community metabolism of an experimental coral reef. Global Biogeochem Cycles 17, 1011.
Lapointe, B. E. (1997), Nutrient threshold for bottom-up control of macroalgal blooms on coral reefs in Jamaica and Southeast Florida, Limnol. Oceanography 42, 1119– 1131.
Leclercq, N., Gattuso, J.P., Jaubert, J., 2000. CO2 partial pressure controls the calcification rate of a coral community. Global Change Biol. 6, 329-334.
Leclercq, N., Gattuso, J.-P., Jaubert, J., 2002. Primary production, respiration,and calcification of a coral reef mesocosm under increased CO2 partial pressure. Limnol. Oceanography 47, 558-564.
Marubini, F., Davies, P.S., 1996. Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. Marine Biol. 127, 319-328.
Marubini, F., Thake, B., 1999. Bicarbonate addition promotes coral growth. Limnol. Oceanography 44, 716-720.
Marubini, F., Atkinson, M.J., 1999. Effects of lowered pH and elevated nitrate on coral calcification. Mar. Ecol. Prog. Ser. 188, 117-121.
Marubini, F., Ferrier-Pages, C., Cuif, J.-P., 2003. Suppression of skeletal growth in scleractinian corals by decreasing ambient carbonate-ion concentration: a cross-family comparison. Proc. R. Soc. Lond. B 270, 179-184.

Marubini, F., Barnett, H., Langdon, C., Atkinson, M.J., 2001. Dependence of calcification on light and carbonate ion concentration for the hermatypic coral Porites compressa. Mar. Ecol. Prog. Ser. 220, 153-162.

Marubini, F., Ferrier-Pages, C., Furla, P., Allemand, D., 2008. Coral calcification responds to seawater acidification: a working hypothesis towards a physiological mechanism. Coral Reefs 27, 491-499.

Mehrbach, C., Culberson, C.H., Hawley, J.E., Pytkowicz, R.M., 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanography 18, 897-907.

Ohde, S., Hossain, M.M.M., 2004. Effect of CaCO3 (aragonite) saturation state of seawater on calcification of Porites coral. Geochem J 38, 613-621.

Pierrot, D., Lewis, E., Wallace, D.W.R. 2006. MS Excel Program Developed for CO2 System Calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee.

Renegar, D.A., Riegl, B.M., 2005. Effect of nutrient enrichment and elevated CO2 partial pressure on growth rate of Atlantic scleractinian coral Acropora cervicornis. Mar. Ecol. Prog. Ser. 293, 69-76.

Reynaud, S., Leclerq, N., Romaine-Lioud, S., Ferrier-Pages, C., Jaubert, J., Gattuso, J.-P., 2003. Interacting effects of CO2 partial pressure and temperature on photosynthesis and calcification in a scleractinian coral. Global Change Biol. 9, 1660-1668.

Rodrigues, L.J., Grottoli, A.G., 2006. Calcification rate and the stable carbon, oxygen, and nitrogen isotopes in the skeleton, host tissue, and zooxanthellae of bleached and recovering Hawaiian corals. Geochim Cosmochim Acta 70, 2781-2789.

Sakka, A., Legendre, L., Gosselin, M., LeBlanc, B., Delesalle, B., Price, N.M., 1999. Nitrate, phosphate, and iron limitation of the phytoplankton assemblage in the lagoon of Takapoto Atoll (Tuamotu Archipelago, French Polynesia). Aquat Microb Ecol 19, 149-161.

Schneider, K., Erez, J., 2006. The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral Acropora eurystoma. Limnol. Oceanogr. 51, 1284-1293.

Tambutte, E., Allemand, D., Bourge, I., Gattuso, J.P., Jaubert, J., 1995. An improved 45Ca protocol for investigating physiological mechanisms in coral calcification. Marine Biol. 122, 453-459.

Tanaka, Y., Miyajima, T., Koike, I., Hayashibara, T., Ogawa, H., 2007. Imbalanced coral growth between organic tissue and carbonate skeleton caused by nutrient enrichment. Limnol. Oceanogr. 52, 1139-1146.

Vandermeulen, J.H., Muscatine, L., 1974. Influence of symbiotic algae on calcification in reef corals: critique and progress report. In: Vernberg, W.B. (Ed.), Symbiosis in the Sea pp.1-19.

Table 1. Carbonate speciation in each treatment. Values are calculated using average source water alkalinity, salinity and input gas pCO_2 values measured during the experiment. Constants of Mehrbach et al. (1973) as refit by Dickson and Millero (1987) are used for carbonate speciation, Dickson (1990) for sulfate. Units are μmol/kg for carbonate species, alkalinity and PO_4, μatm for pCO_2. An Excel implementation of CO2sys (Pierrot et al., 2006) was used for calculations.

treat-ment	T (^oC)	S	PO_4	Alk	pCO_2	pH total	CO_2	HCO_3	CO_3	Ω aragonite
ambient	25.8 (.1)	31 (.9)	0.5 (.3)	2110 (40)	390 (10)	8.03	11	1662	182	3
CO_2	25.8 (.1)	31 (.9)	0.5 (.3)	2110 (40)	780 (40)	7.78	22	1837	111	1.8
nutrient	25.8 (.1)	31 (.9)	0.7 (.2)	2110 (40)	390 (10)	8.03	11	1662	182	3
nutrient & CO_2	25.8 (.1)	31 (.9)	0.7 (.2)	2110 (40)	780 (40)	7.78	22	1837	111	1.8

Table 2. Factorial ANOVA results, showing the significance of CO_2, nutrients, and their interaction on rank transformed calcification rates with the parent coral colony included as a blocking variable

Source	DF	SS	Mean Square	F	p
Model	7	620.7	88.7	2.68	0.0485
Error	16	529.3	33.1		
CO2	1	216	216	6.53	0.0212
nutrients	1	4.17	4.17	0.13	0.7273
CO2*nut	1	140.17	140.17	4.24	0.0562
colony	4	260.4	65.1	1.97	0.1482

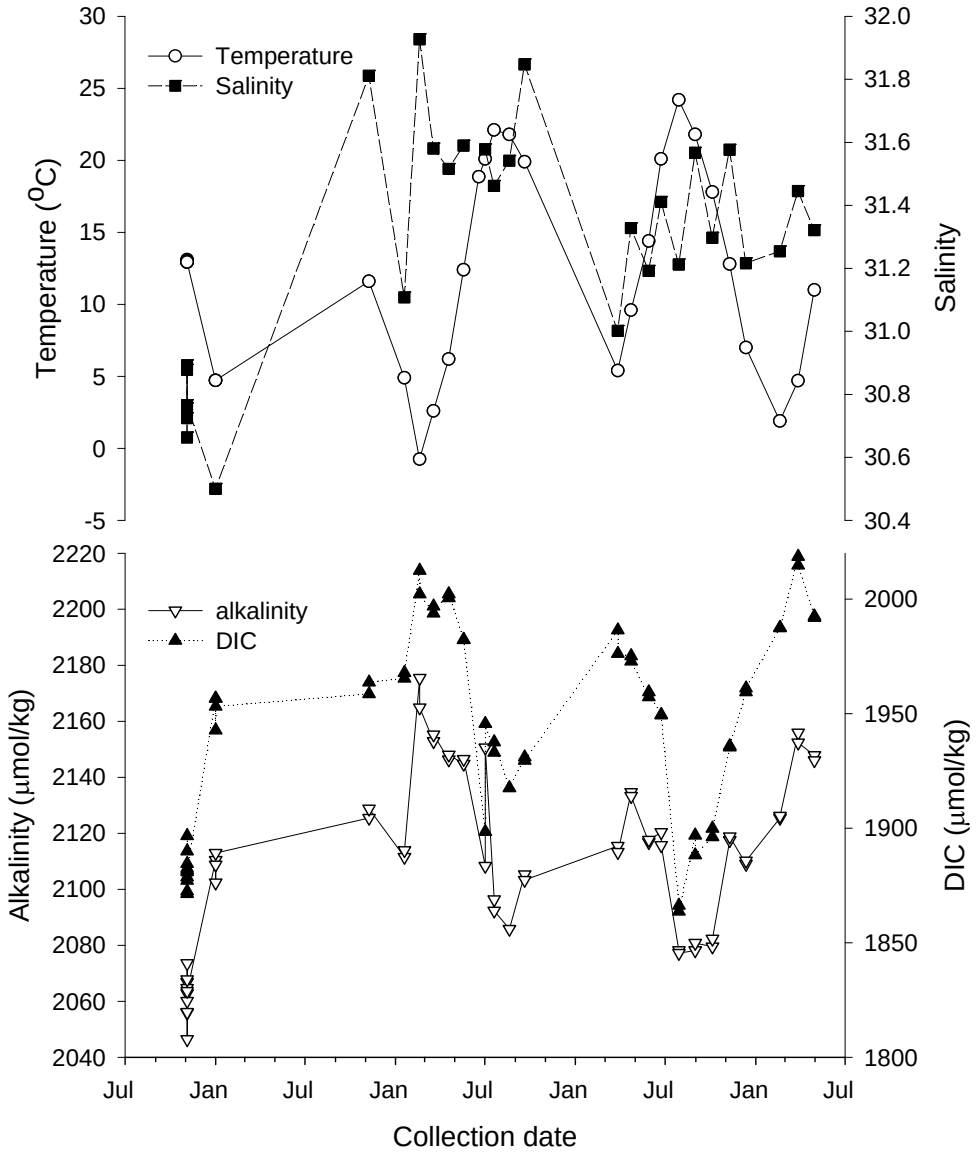


Figure 1. Temperature, salinity, alkalinity and DIC, values for samples collected 2005-2009 from Great Harbor, Woods Hole, MA. This shows the natural range in carbonate chemistry to which the *A. poculata* colonies used in this experiment are exposed. T and S are plotted in the upper plot, alkalinity and DIC in the lower. Points are individual measurements (usually 2 samples were taken at each time point). Values and dates are provided as a supplementary table.

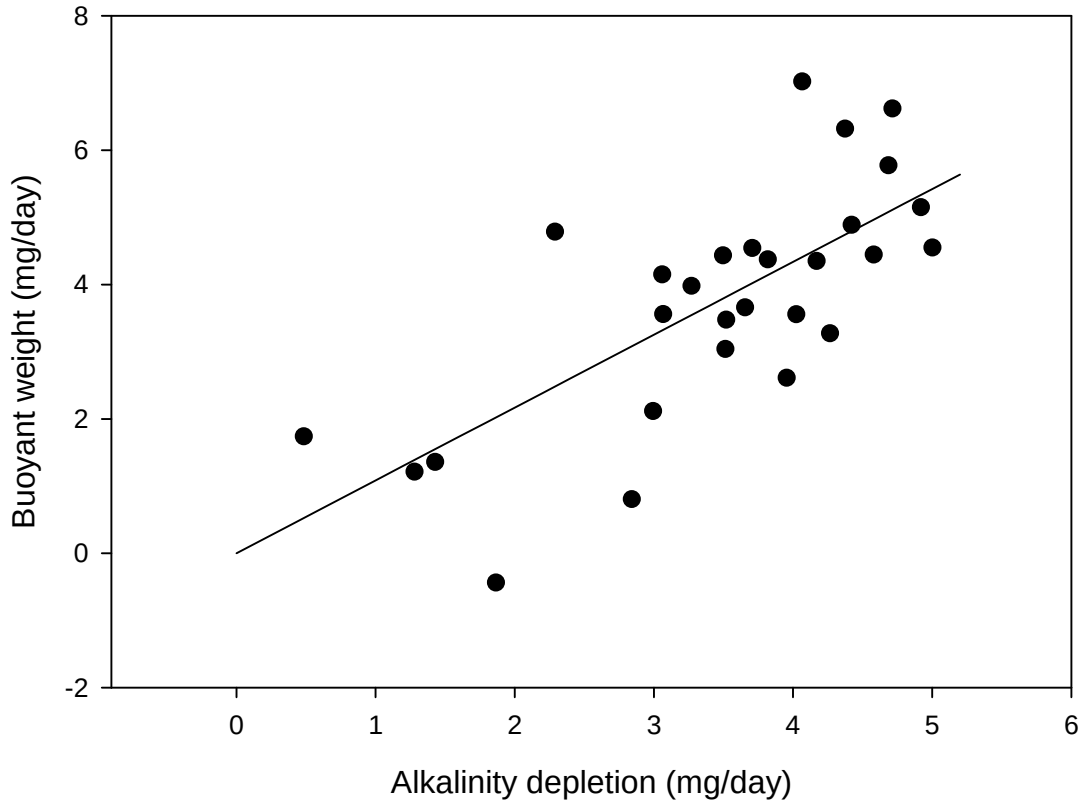


Figure 2. Relationship between growth rates based on alkalinity depletion and buoyant weight measurements. Each point represents a measurement for an individual pair of corals, the line represents the best fit line (buoyant weight = 1.084* alkalinity depletion, R^2=0.523).

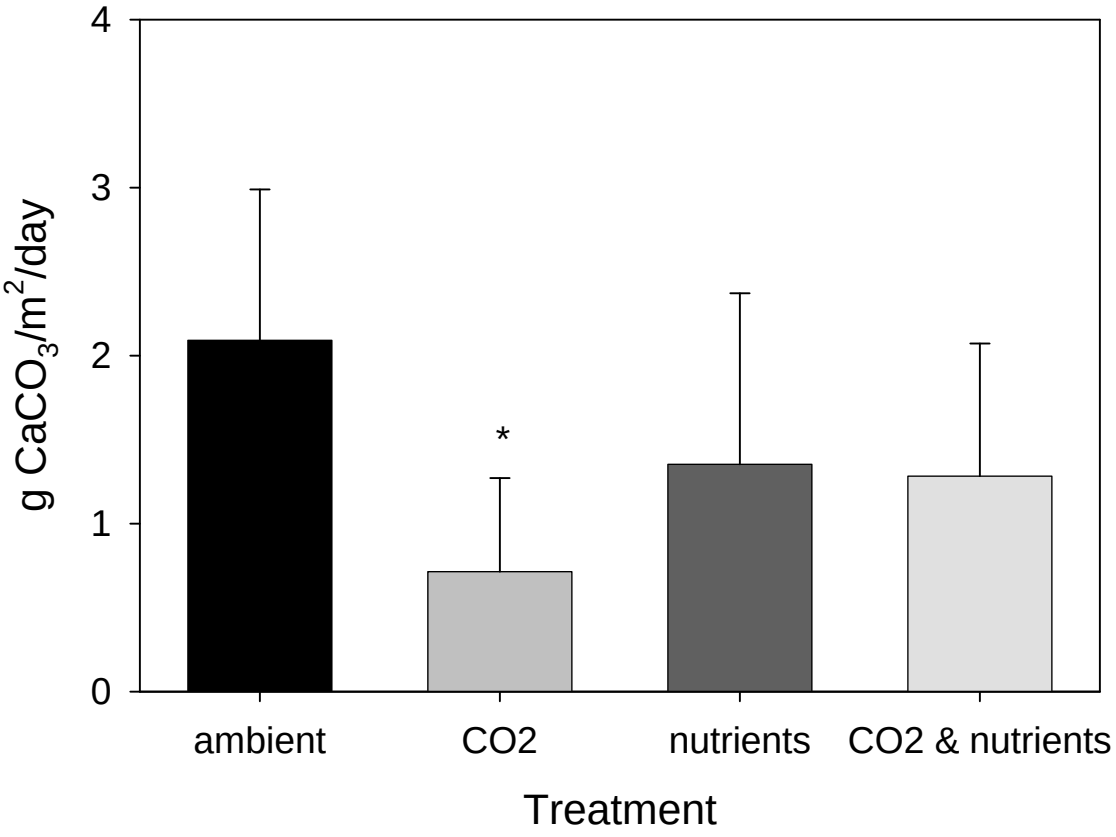


Figure 3. Average growth rates of zooxanthellate *A. poculata* colonies exposed to nutrient and CO_2 treatments for six months. Bars represent means of six replicates, error bars are standard deviation. * indicates treatments significantly different from ambient $p<0.05$.

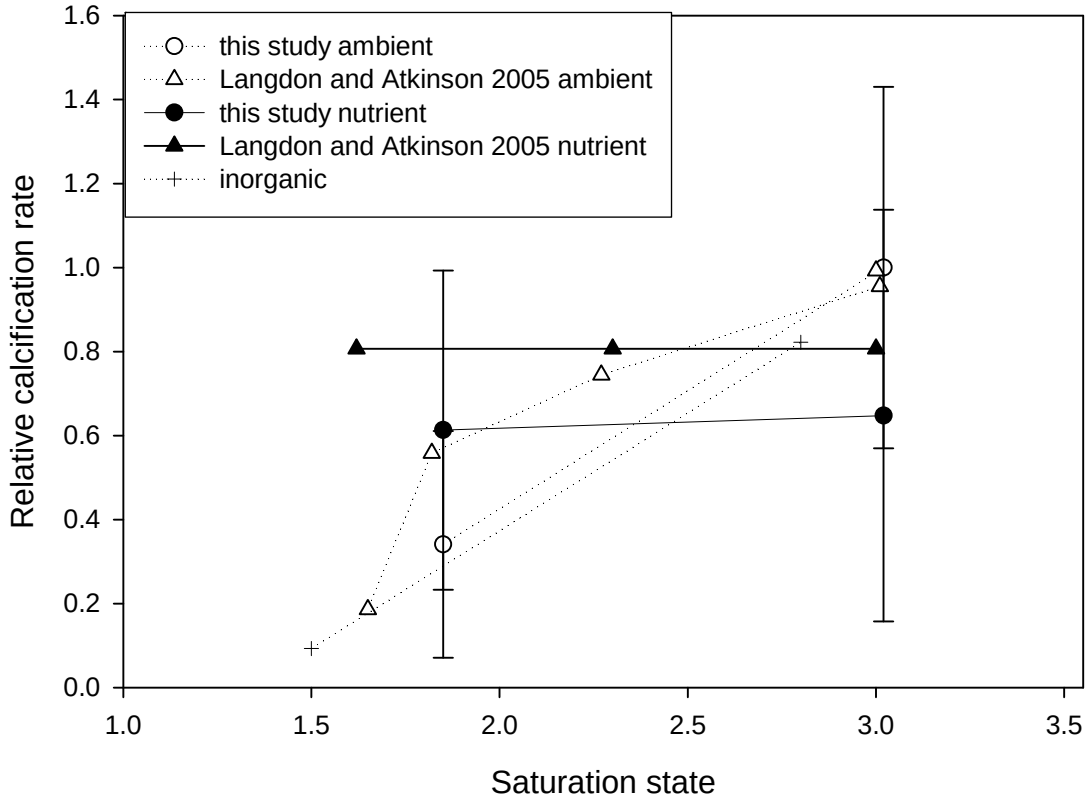


Figure 4. Comparison of relative calcification rates versus saturation state measured in the current study (circles) and values reported by Langdon and Atkinson (2005) (triangles). Corals are grown under either ambient nutrients (open symbols) or elevated nutrients (filled symbols). For clarity, error bars are only included for the current study. Inorganic aragonite growth data from Burton and Walter (1987) (cross) are included for reference purposes. Relative calcification rates are expressed as the ratio of the reported growth rate to the calculated growth rate under ambient conditions at a saturation state of 3 (values at 3 were calculated assuming a linear relationship between saturation state and growth using the 2 saturation states closest to 3).

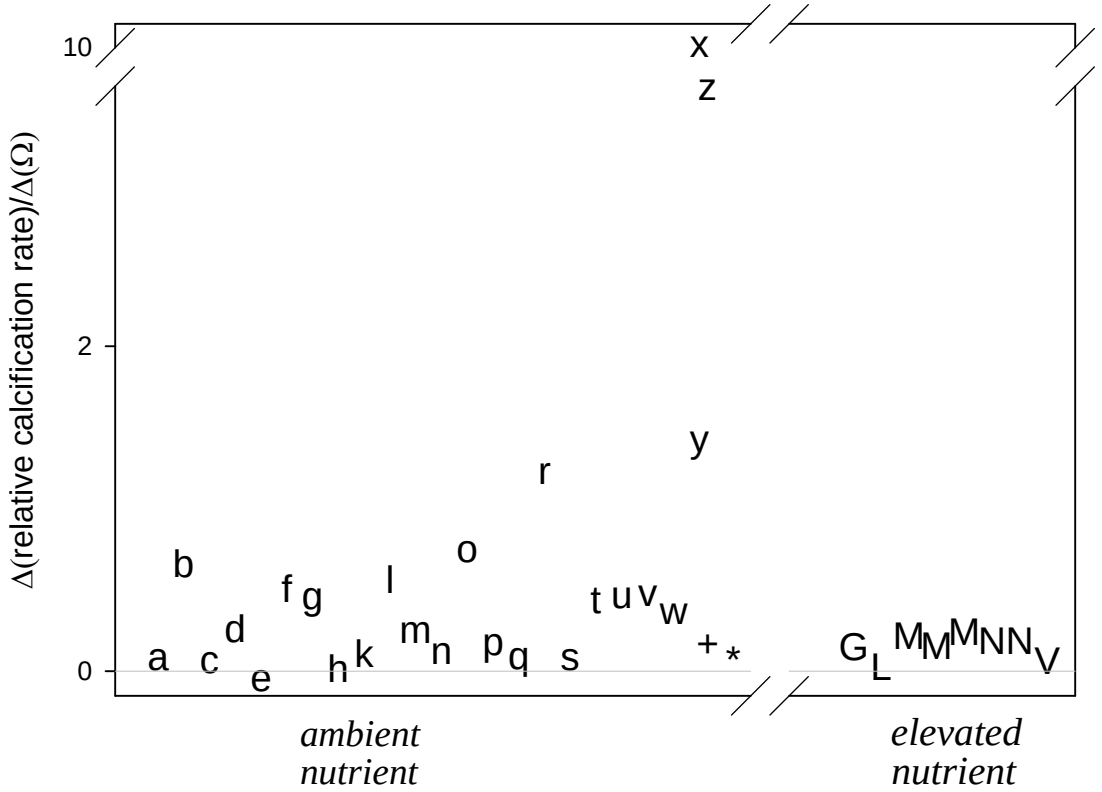
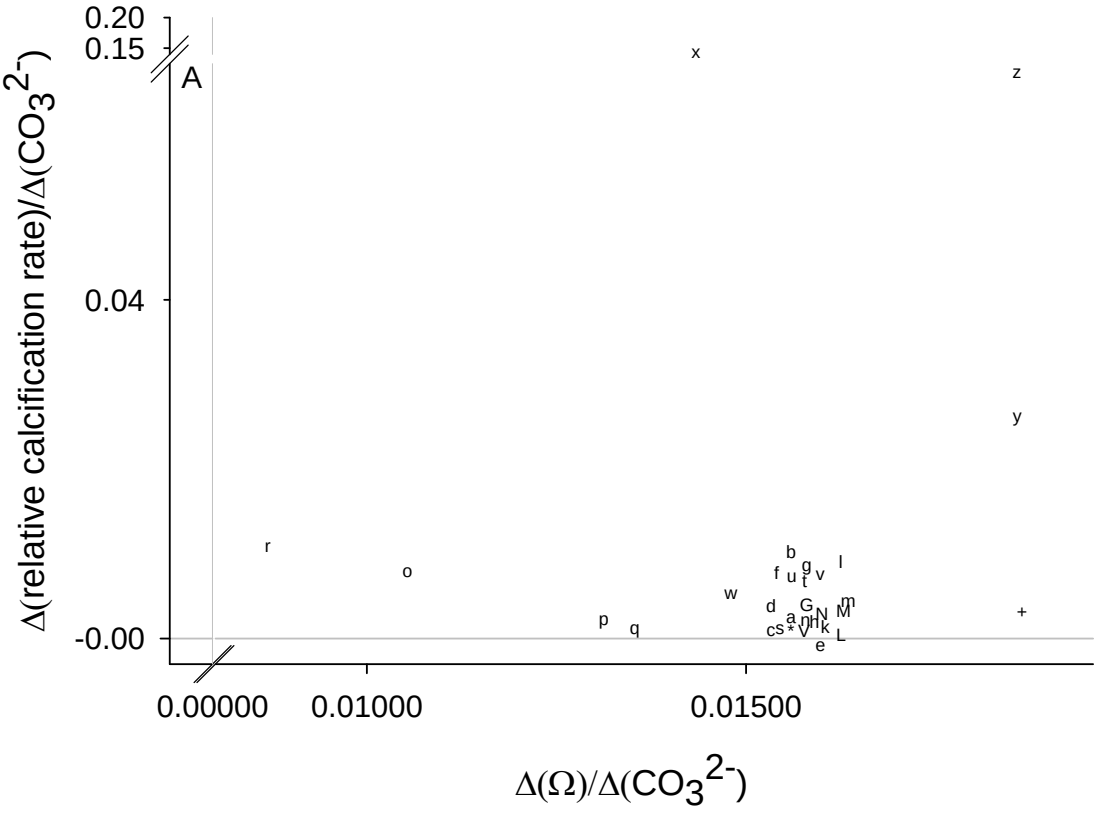


Figure 5. Changes in the relative calcification rate in response to saturation state for ambient and nutrient enriched conditions. Positions along the x-axis within the ambient and nutrient enriched groups are arbitrary and intended only to separate symbols. Y-axis values are slopes from linear regressions of relative calcification rate versus saturation state determined for each study. Higher Y values indicate a positive slope, and thus decreasing calcification rate with decreasing saturation state. Near-zero Y values indicate little response of calcification to lowered saturation state. Each letter represents a different study: letters a-l represent studies in which CO_2 was manipulated, the remainder used acid/base and/or bicarbonate addition. Some studies used multiple nutrient levels, different levels are represented by the same letter, not all studies included nutrients as a treatment and are thus plotted at ambient nutrients only. Most studies showed significant correlations ($p<0.1$) between relative calcification rate and saturation state, studies with correlations of $p>0.3$ are excluded (using a p value of 0.3 allows inclusion of studies h and k which were not well fit by a linear relationship but for which there was a response to saturation state). When data ranges were given instead of average values, the minimum and maximum were averaged for calculations. Studies are as follows: a = Leclerq et al., 2000 light, b = Leclerq et al., 2000 dark, c = Leclerq et al., 2002 light, d = Leclerq et al., 2002 dark, e = Reynaud et al., 2003 25^{o}C, f = Reynaud et al., 2003 28^{o}C, g = Renegar and Riegl, 2005, h = Anthony et al., 2008 *Porites sp.* ~25.5^{o}C, i = Anthony et al., 2008 *Porites sp.* ~28.5^{o}C, j = Anthony et al., 2008 *Acropora sp.* ~28.5^{o}C, k = Anthony et al., 2008 *Acropora sp.* ~25.5^{o}C, l = This study, m = Marubini and Atkinson, 1999, n = Marubini and Thake, 1999, o = Langdon et al., 2000, p = Marubini et al., 2001, q = Marubini et al., 2001, r = Langdon et al., 2003, s = Marubini et al., 2003, t =

Ohde and Hossain, 2004 dark, u = Ohde and Hossain, 2004 light, v = Langdon and Atkinson, 2005, w = Schneider and Erez, 2006, x = Schneider and Erez, 2006 dark, y = Herfort et al., 2008 *Acropora sp.* dark, z = Herfort et al., 2008 *Acropora sp.* light, + = Herfort et al., 2008 *Porites sp.*, * = Marubini et al., 2008.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0.00000
0.01000
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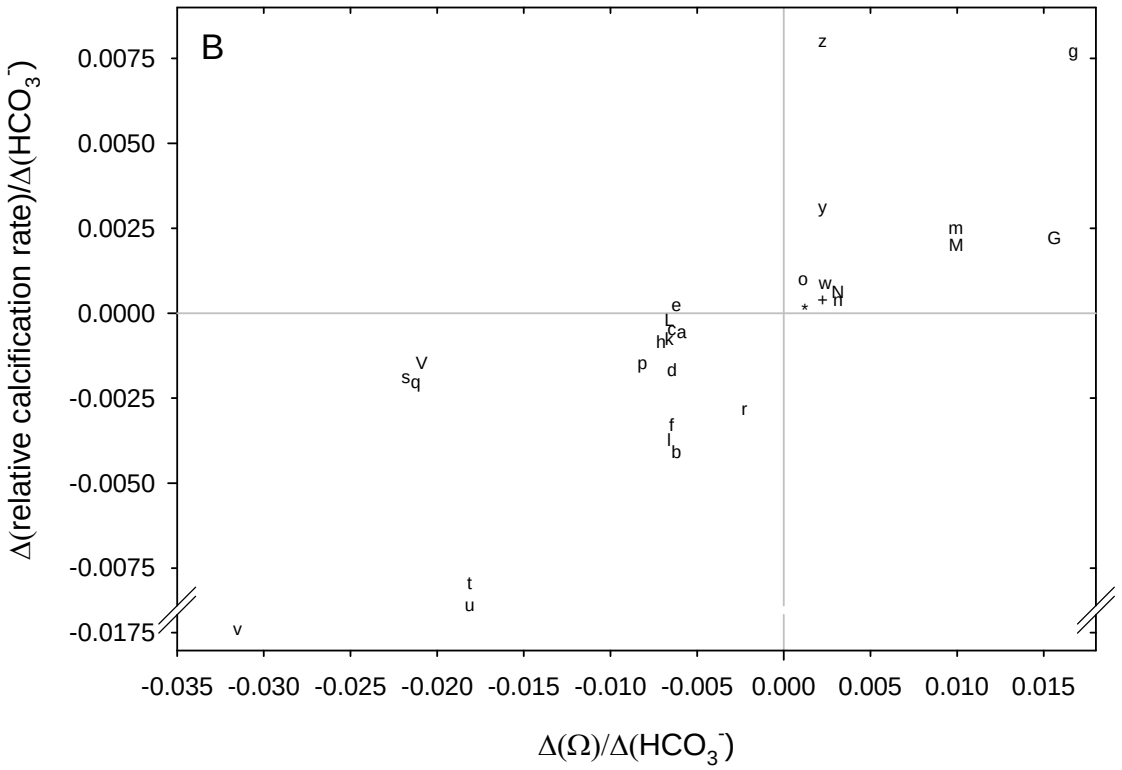
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Δ(relative calcification rate)/Δ(HCO3-)
Δ(Ω)/Δ(HCO3-)
z
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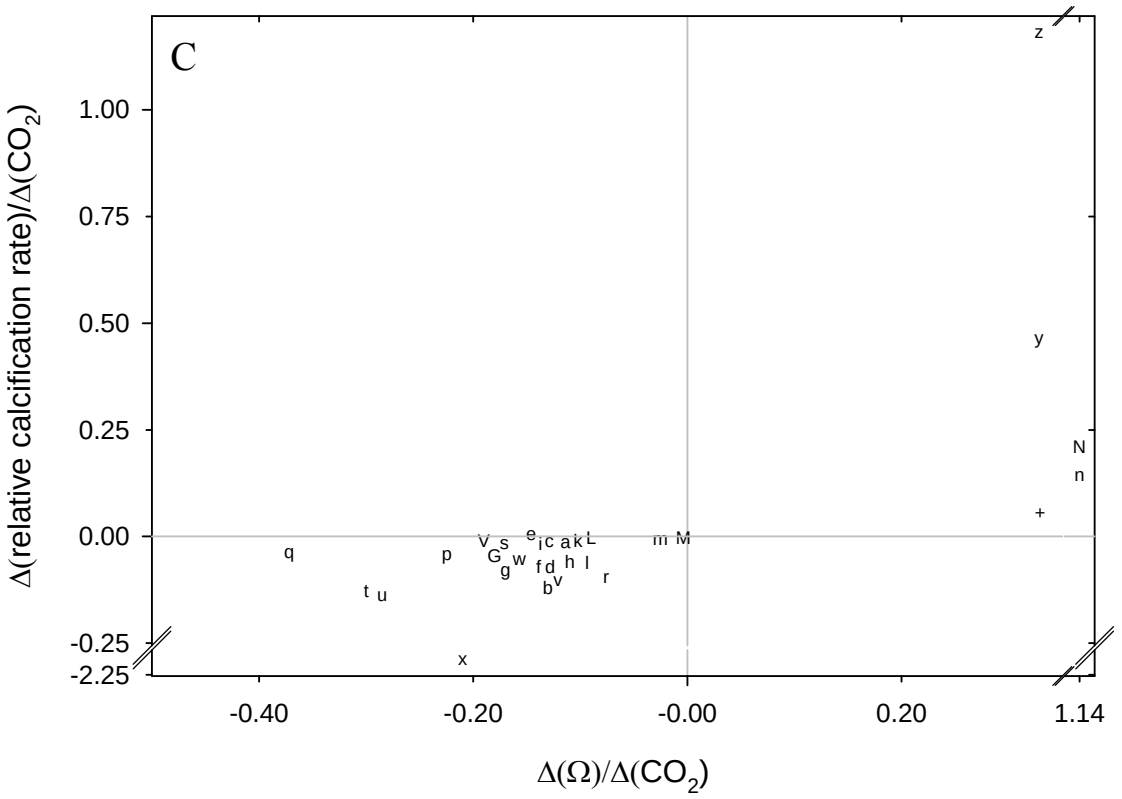
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Δ(relative calcification rate)/Δ(CO2)
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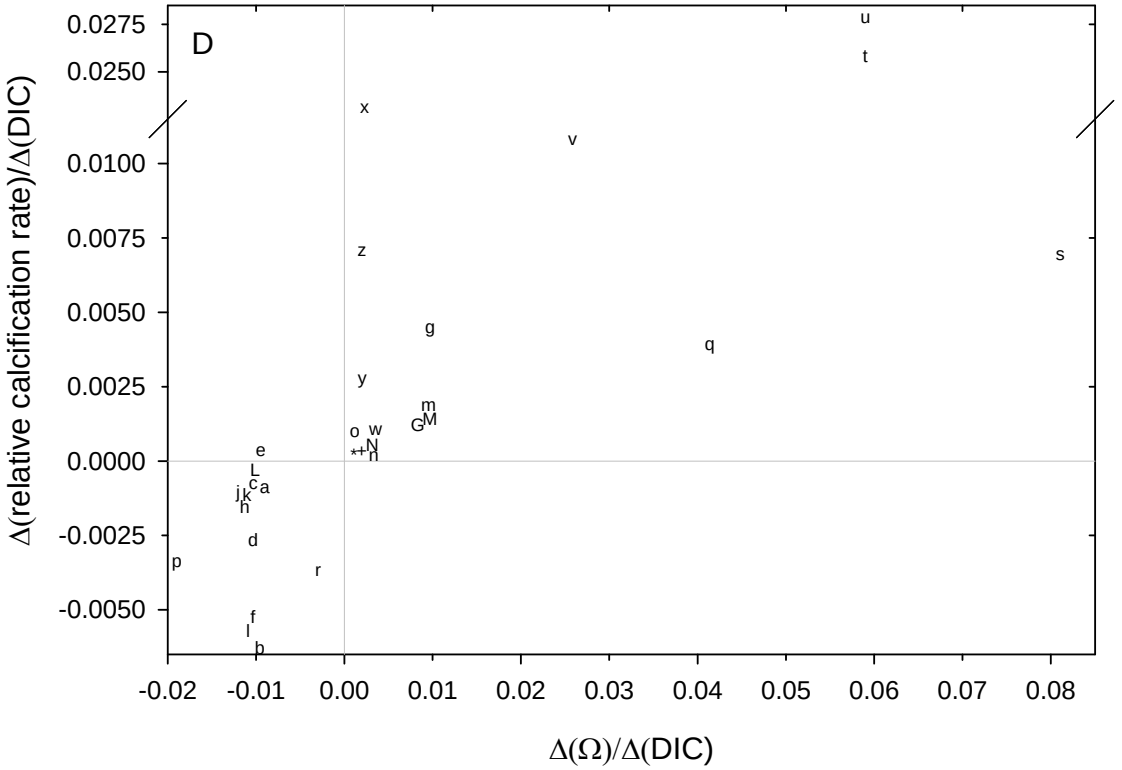

Figure 6. Slope-slope plots of changes in the relative calcification rate as a function of the concentration of carbonate species (carbonate ion, bicarbonate ion, aqueous CO_2 and total DIC), versus the change in saturation state as a function of the carbonate species concentration. A. Change in the relative calcification rate in response to carbonate ion versus the change in saturation state with carbonate ion. Note that in all cases, saturation state and carbonate-ion concentrations are positively correlated, and in all but one case, calcification is positively correlated with carbonate-ion. As a result, all but one study falls in the upper right quadrant. This pattern is consistent with calcification rate being controlled by either carbonate ion concentration or saturation state. B. Changes in the relative calcification rate in response to bicarbonate ion versus the change in saturation state with bicarbonate ion. Note that in all cases in which saturation state and bicarbonate-ion concentrations are positively correlated, calcification is positively correlated with bicarbonate-ion (points fall in the upper right quadrant). However, when saturation state and bicarbonate are negatively correlated, calcification is generally negatively correlated with bicarbonate (points fall in lower left quadrant). This pattern is not consistent with a bicarbonate ion concentration control on calcification rate, instead saturation state appears to be the controlling factor. C. Changes in the relative calcification rate in response to aqueous CO_2 versus the change in saturation state with aqueous CO_2. Note that in all cases in which saturation state and aqueous CO_2 concentrations are positively correlated, calcification is positively correlated with aqueous CO_2 (upper right quadrant). When saturation state and aqueous CO_2 are negatively correlated, calcification is generally negatively correlated with aqueous CO_2 (lower left quadrant). As with the bicarbonate data, this pattern suggests the key role of

saturation state in controlling calcification rate. D. Changes in the relative calcification rate in response to DIC versus the change in saturation state with DIC. Here too, the pattern suggests that saturation state, and not DIC concentration per se, controls calcification rate. Y-axis values are slopes from linear regressions of relative growth rate versus the given carbonate species determined for each study. X-axis values are from linear regressions of saturation state versus the given carbonate species. For carbonate species not listed in the original publication, concentrations were calculated using CO2sys (Pierrot et al., 2006), with the reported measured values as inputs. The constants of Mehrbach et al. (1973) are used for carbonate speciation, Dickson (1990) for sulfate. All plotted studies showed correlations ($p<0.3$) between relative calcification rate and the given carbonate species and between saturation state and the given carbonate species. Overlapping symbols are offset. Each letter represents a different study as in Figure 5. Nutrient enriched conditions are indicated by a capital letter.

Supplementary Materials

Table 1. Average alkalinity (µmol/kg), DIC (µmol/kg), temperature (^{o}C) and salinity values for samples collected 2005-2009 from Great Harbor, Woods Hole, MA at ~1.5m depth. Replicate alkalinity and DIC measurements differed by less than 11 µmol/kg except for samples collected 7/2/07, when reproducibility was better than 50 µmol/kg.

Date	Temperature	Salinity	Alkalinity	DIC
11/4/05 14:30	13.1	30.73	2051	1877
11/4/05 22:44	13.0	30.76	2066	1883
11/5/05 6:40	12.9	30.88	2071	1893
11/5/05 11:15	12.9	30.66	2059	1874
11/5/05 15:15	12.9	30.77	2062	1880
1/1/06 10:30	4.7	30.50	2110	1957
1/1/06 18:00	4.7	30.50	2108	1948
11/8/06 14:00	11.6	31.81	2127	1961
1/19/07 16:00	4.9	31.11	2113	1967
2/19/07 17:00	-0.8	31.93	2170	2007
3/19/07 16:10	2.6	31.58	2154	1995
4/19/07 16:40	6.2	31.52	2147	2001
5/19/07 18:00	12.4	31.59	2146	1982
7/2/07 17:50	20.1	31.58	2129	1922
7/20/07 17:50	22.1	31.46	2094	1935
8/20/07 17:00	21.8	31.54	2086	1918
9/20/07 18:10	19.9	31.85	2104	1930
3/27/08 17:30	5.4	31.00	2114	1981
4/23/08 17:00	9.6	31.33	2134	1974
5/29/08 16:30	14.4	31.19	2117	1958
6/23/08 17:50	20.1	31.41	2118	1949
7/29/08 17:50	24.2	31.21	2078	1865
8/31/08 18:08	21.8	31.57	2080	1893
10/5/08 18:05	17.8	31.30	2081	1898
11/9/08 18:40	12.8	31.58	2118	1936
12/12/08 19:09	7.0	31.22	2110	1960
2/19/09 20:30	1.9	31.25	2126	1987
3/28/09 18:12	4.7	31.45	2154	2017
4/30/09 18:00	11.0	31.32	2147	1992